Intracellular drug delivery: aing cross presentation of subunit vaccines

Last Time: basic vaccine concepts

Today: Using synthetic biomaterials to enhance cytosolic delivery of molecules

Reading: Wang et al. ‘Moleculaely engineered poly(ortho ester) microspheres for enhanced delivery of DNA vaccines,’ Nat. Mater. 3 190-196 (2004)

Supplementary Reading:

ANNOUNCEMENTS:

Course Evaluation Next Tuesday 5/16
Particle-stimulated cross presentation

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INTRACELLULAR DRUG DELIVERY AND VACCINES:

1. Boost cross presentation of protein antigens
2. Cytosolic delivery of DNA is a step on path to nucleus

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ENDOSOMAL ESCAPE:

Enhancing cross presentation
cytosolic delivery of large macromolecules

(1) ‘proton sponge’ effect
(2) pH-activated polymers
    and peptides
INTRACELLULAR VS. EXTRACELLULAR ENVIRONMENT

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Proton sponge effect

**ENDOSOMAL ESCAPE: 'PROTON SPONGES'**

1. **Osmotic swelling that bursts** OR causes endosome to become leaky
2. **Proton pumps** try to keep pH constant
3. **Anion flux** to maintain charge neutrality
4. **Vector** becomes a high-capacity proton sink

**CAVEATS:**
Polyocations/cationic lipids tend to be toxic at high doses

**ENDOSOMAL ESCAPE:**

- **NaCl**
- **H₂O**
- **Cytoplasm**
- **Ion transporters**
- **Proton pumps**
- **Polyethyleneimine**
Role of additional structural features of PEI in efficient endosomal escape:

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Endosomal escape by direct membrane interactions

Both polycation and polyanion headgroups with pKas = 5-7 can promote endosomal escape:

ENDOSOMAL ESCAPE:
PH-RESPONSIVE POLYMERS

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pH: 7.4

Phase transitions driven by:
- Concentration, Temp, pH

Phase transition causes fusion with vesicle membrane

Protonation/uncharging

Polyanionic liposome

pH: 5.0

Lecture 21 Spring 2006
Endosomal escape by direct membrane interactions

Both polycation and polyanion headgroups with pKas = 5-7 can promote endosomal escape:

pH: 7.4

Polycationic liposome

pH: 5.0

Electrostatic attraction of liposome to vesicle wall
STRATEGIES FOR CUED ‘BURST’ RELEASE OF CARGO COINCIDENT WITH ENDOSONMAL ESCAPE

ENDOSOMAL ESCAPE: PH-RESPONSIVE POLYMERS

POLY (β-AMINO ESTERS)

CHARGED AT pH ≤ 7

BIODEGRADABLE

PBAE MICROSPHERE LOADED W/DRUG

POLYMER DISSOLVES

RAPID + TOTAL RELEASE OF DRUG

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Approaches to endosome escape:
‘encrypted’ polymers

Multi-function molecular carriers:

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ENDOSOMAL ESCAPE: PH-RESPONSIVE POLYMERS

(1) RECEPTOR TARGETING

(2) DRUG TARGETING MECHANISM

(3) PH DECREASE

(4) CYTOSOLIC REDUCTION
Bioinspired pH-Responsive Polymers for the Intracellular Delivery of Biomolecular Drugs."

Results with peptide delivery by encrypted polymers

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Example vaccine results: pH-responsive gels as vaccines

Example vaccine results: pH-responsive gels as vaccines

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DIRECT ENTRY TO THE CYTOSOL

Membrane-penetrating peptides

Pore-forming peptides
(DERIVED FROM PATHOGENS)

Fusogenic peptides
(LIPOSOME)
(DERIVED FROM VIRUSES)
Cell-penetrating peptides (CPPs) [aka Protein Transduction Domains (PTDs)]


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DIRECT ENTRY TO CYTOSOL:
MEMBRANE-PENETRATING PEPTIDES

Sources and sequences

CIPS TEND TO HAVE:

{HYDROPHOBIC SEQUENCE

CATIONIC: HIS (H) LYS (K) ARGinine (R)

HYDROPHOBIC: ALA (A) VAL (V) TRP (W) ...

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Penetratin:
Short peptide sequence from drosophila transcription factor protein Antennapedia

RQIKIWFQNRRMKWKK

Models of membrane-penetrating peptide function

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Please see: Figure 7 in Derossi, D., et al.
CPP function in vitro

DIRECT ENTRY TO CYTOSOL: MEMBRANE-PENETRATING PEPTIDES

Uptake of penetratin by primary neuronal cells:

- **Caveat 1:** Typically can deliver peptides up to 100 aa in length \( \rightarrow \) \( <60 \text{ kDa} \)
- **Caveat 2:** Some question about universality - work in all cells?

Protein delivery using HIV tat peptide:

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ACTIVATION ON ENTRY TO THE CYTOSOL
Selective bond dissociation using reversible disulfide linkages

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Pore-forming proteins/peptides as a tool for membrane-penetrating drug carriers

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Please see: Figure 1 in Bhakdi, S., et al.
"Staphylococcal Alpha-Toxin, Streptolysin-O and Escherichia Coli Hemolysin: Prototypes of Pore-Forming Bacterial Cytlysins."
DIRECT ENTRY TO CYTOSOL: FUSOGENIC PEPTIDES

fusogenic peptides: using viral entry strategies for drug delivery

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Further Reading